

## REMARKS

Applicants request reconsideration of the February 13, 2003 Office Action based upon the following comments.

### *Amendments*

Claim 1 was amended to clarify the subject matter of the claimed invention.

Claim 6 was amended to place it in independent (and allowable) form. The Examiner indicated that this claim was "free from prior art".

Claims 24 was amended to address a potential 112 issue regarding the ranges set forth in the claim.

Claim 30 and 32 were amended for minor grammatical purposes.

Claim 35 was added to the application and covers one of the preferable range that was formerly set forth in claim 24.

### *Claim Rejection-35 U.S.C § 103*

Independent claims 1 and dependent claim 33 are rejected as obvious based upon Walkenbach [IDS-12, AO, 1991], Ingemansson I [IDS-12, AN, 1995], and Ingemansson II [IDS-1, AT-2, 1995] in further view of Pinsky [IDS-1, AQ-2, 1994] and Naka [IDS-1, AT-1, 1995].

The Examiner admits that Walkenbach, Ingemansson I, and Ingemansson II fail to disclose the claimed nitroglycerin limitation set forth in independent claim 1. The Examiner attempts to alleviate this failing based upon the teachings of Pinsky and Naka. The Examiner suggests that the motivation for the proposed combination is based on the belief that

nitroglycerin is allegedly known to enhance survival of animal tissue and maintained vascular homeostasis. The Examiner asserts that the suggested amount of nitroglycerin is about 0.1 mg/ml.

Applicants remind the Examiner that the inventor Stig Steen has provided a signed declaration that should be reviewed by the Examiner. Stig Steen is one of the authors of the Ingemansson I and Ingemansson II references. The declaration sets forth what one skilled in the art would understand to be a genuine “preservation solution” and the state of the art at the time of the invention.

Applicants have amended claim 1 to incorporate the features described on page 25 of the application and set forth in claim 30, namely that “nitroglycerin is present in an amount of about  $10^{-4}$  -  $10^{-7}$  M; and calcium ion is present in an amount of about 0.3 - 1.5 mM, based on the final volume of preservation solution”.

Applicants respectfully submit that claim 1 is allowable in view of the cited prior art. The cited prior art fails to suggest the unique combination of calcium ions, at least one colloidosmotically active substance, and nitroglycerine as set forth in claim 1. The Walkenbach, Ingemansson I, and Ingemansson II patents fail to provide any teaching, hint, or suggestion regarding the use of nitroglycerin. Applicants also assert that Pinsky and Naka fail to teach the use of nitroglycerin in a solution that also contains calcium ions and/or a colloidosmotically active substance. For example, Applicants assert that Pinsky and Naka teach the use of nitroglycerin in Ringer’s solution (which does not contain a colloidosmotically active substance as defined by the Applicants).

It is Applicants’ position that the cited prior art fails to provide motivation for the combination set forth in the Office Action. Motivation for a proposed combination must be

factually supported based upon objective evidence of record. This objective evidence must establish that the cited prior art provides some motivation, suggestion, or teaching regarding the desirability of making the specific combination. The factual question of motivation is material to patentability, and can not be resolved on subjective belief and unknown authority. In re Sang Su Lee, 277 F.3d 1338, 61 USPQ2d 1430 (Fed. Cir. 2002).

As such, Applicants respectfully submit that claim 1 and 33 are allowable and the rejection should be withdrawn.

Applicants wish to provide further comments regarding the synergistic effect set forth in the present application. The effect of combining calcium and nitroglycerine is synergistic in the sense that these both components have contrasting activities, i.e. calcium has a contracting activity on the endothelium and nitroglycerine a relaxing activity. This combination leads to the advantageous effect that the calcium, which binds together the sugar moieties in the glycocalix layer of the endothelium, is maintained in the glycocalix layer, i.e. is not escaping to the extracellular environment, whereby the integrity of the endothelium remains intact and reduced endothelium function is avoided. The presence of nitroglycerine in such a solution relaxes both the smooth muscles and thereby the endothelium, thus avoiding detrimental cold induced cracking of the endothelium and, at the same time, forces intracellular calcium ions through the endothelium out to the extracellular environment or into sarcoplasmic or cytoplasmic reticuli within the cell. This unique combination gives the advantageous and unforeseeable effects of relaxation of all structures in the vessel wall combined with a maintained endothelial integrity. This is a substantial improvement in relation to current long-term preservation techniques.

Fig. 1 reflects one aspect of the improvements obtained with the present invention, i.e. the <sup>contraction</sup> concentration induced on rat aorta after the storage during 48 hours. In the preservation solution according to the present invention (the <sup>Bar</sup> staple most to the right) the contraction is regarded as normal (i.e. 100%). A Perfadex solution further comprising only calcium gives subnormal <sup>not true</sup> contraction as indicated with the staple in the middle. A preservation solution that contains only nitroglycerine induces an even more sub-normal contraction. This shows that a preservation solution according to the present invention maintains the contractile performance at the normal value after storage during 48 hours, i.e. that a desired preservation of the vessel has taken place.

Calcium is the final transmitter substance eliciting vascular smooth muscle contraction in blood vessels. In a relaxed vessel, the intracellular calcium concentration around the contractile proteins actin and myosin is low. The intracellular calcium ions are kept “locked into” the sarcoplasmatic reticulum by active calcium pumps pumping the calcium ions into these “storage house” of the cell. If a blood vessel is cooled down to 4°C, the efficiency of the calcium pumps decreases, and the intracellular concentration of the calcium increases thereby causing a cold-induced vasoconstriction. Nitroglycerin blocks this cold-induced vasoconstriction by keeping the intracellular concentration of calcium low (thereby causing vasodilation). The glycocalix layer covering the vascular endothelium extracellularly is kept intact by help of calcium ions incorporated in this layer. If vascular endothelium is exposed to a solution without calcium, calcium from the glycocalix layer start to diffuse into the calcium free medium. The protecting glycocalix layer thereby disintegrate and the endothelium will gradually loose its integrity.

It should also be noted that Claim 1 sets forth particular ranges for the nitroglycerin and calcium ions. The results of Figure 1 fall with these claimed ranges. Furthermore, a “colloidosmotically active substance” has a particular definition as set forth on page 21 of the

application. Contrary to the assertions in the Office Action, water would not be considered a "colloidsmotically active substance" as set forth in the present application. Whereas, Perfadex includes a "colloidsmotically active substance" as set forth in the application (refer to page 24 of the application for details regarding Perfadex).

Due to the foregoing, Applicants respectfully submit that the aforementioned synergism is shown and probative of the claims being non-obviousness.

### CONCLUSION

Applicants respectfully requests allowance of the application. If any additional fees are due in connection with the filing of this response, such as fees under 37 C.F.R. §§ 1.16 or 1.17, please charge the fees to Deposit Account No. 02-4300. Any overpayment can be credited to Deposit Account No. 02-4300.

Respectfully submitted,

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\* Practice is limited to matters and proceeding before federal courts and agencies.